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ABSTRACT

The research projects during this period were focused on the oxidation chemistry of singlet molecular oxygen and on the development of efficient chemiluminescent systems. Major accomplishments of our investigations include the following:

(1) The first heterogeneous photosensitizer for the photochemical production of singlet oxygen in organic solvents was prepared. This material has since been produced commerically and sold under the tradename SENSITOX. Additional sensitizers have been synthesized for use in aqueous systems.

(2) 1-Phospha-2,8,9-trioxaadamantane ozonide was found to decompose at ambient temperature to provide a convenient chemical source of singlet oxygen.

(3) A new method for generation of chemiluminescence by the silica-catalyzed rearrangement of thermally stable endoperoxides to 1,2-dioxetanes was discovered in our laboratory.

(4) A procedure for obtaining dramatic increases in the chemiluminescence efficiency of dioxetanes by employing silica catalysis was developed.

(5) A study of substituent effects on the decomposition of 1,2-dioxetanes provided new insight into the mechanism of light production by these peroxides.

(6) A new technique for triggering the chemiluminescent decomposition of a stable 1,2-dioxetane was investigated.

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TABLE OF CONTENTS

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PUBLICATIONS ACKNOWLEDGING ARO SUPPORT

1. A. P. Schaap and G. R. Faler, Mechanism of 1,2-Cycloaddition of Singlet Oxygen to Alkenes. Trapping of Perepoxide Intermediate, *J. Am. Chem. Soc., 95,* 3381 (1973).

2. A. P. Schaap and G. R. Faler, A Convenient Synthesis of Adamantylidene-adamantane, *J. Org. Chem., 38,* 3061 (1973).

3. K. Goda, J. Chu, T. Kimura, and A. P. Schaap, Cytochrome C Enhancement of Singlet Molecular Oxygen Production by the NADPH-Dependent Adrenodoxin Reductase-Adrenodoxin System: The Role of Singlet Oxygen in Damaging Adrenal Mitochondrial Membranes, *Biochem. Biophys. Res. Commun., 52,* 1300 (1973).

4. E. C. Blossey, D. C. Neckers, A. L. Thayer, and A. P. Schaap, Polymer-Based Sensitizers for Photooxidations, *J. Am. Chem. Soc., 95,* 5820 (1974).

5. A. P. Schaap, A. L. Thayer, G. R. Faler, K. Goda, and T. Kimura, Singlet Molecular Oxygen and Superoxide Dismutase, *J. Am. Chem. Soc., 96,* 4025 (1974).

6. K. Goda, T. Kimura, A. L. Thayer, K. Kees, and A. P. Schaap, Singlet Molecular Oxygen in Biological Systems: Non-Quenching of Singlet Oxygen-Mediated Chemiluminescence by Superoxide Dismutase, *Biochem. Biophys. Res. Commun., 58,* 660 (1974).

7. A. P. Schaap, K. Kees, and A. L. Thayer, 1-Phospha-2,8,9-trioxadamantane Ozonide: A Convenient Source of Singlet Molecular Oxygen, *J. Org. Chem., 40,* 1185 (1975).

8. A. P. Schaap, A. L. Thayer, E. C. Blossey, and D. C. Neckers, Polymer-Based Sensitizers for Photooxidations II, *J. Am. Chem. Soc., 97,* 3471 (1975).

9. N. J. Turro, G. Schuster, H. C. Steinmetzer, A. P. Schaap, G. R. Faler, and W. Adam, Adamantylideneadamantane1,2-Dioxetane. An Unusual 1,2-Dioxetane Decomposition Mechanism, *J. Am. Chem. Soc., 97,* 7110 (1975).

10. A. P. Schaap, A. L. Thayer, and K. Kees, *cis*-Dimethoxy-1,2-Dioxetane, *Organic Photochemical Syntheses, vol. II,* p. 49, Wiley Interscience, New York, 1976.

11. A. P. Schaap, K. Goda, and T. Kimura, Production of Singlet Molecular Oxygen by the Adrenodoxin Reductase-Adrenodoxin Enzyme System, In the *Excited States of Biological Molecules,* Wiley & Sons, New York, p. 79 ((1976).

12. A. P. Schaap, A. L. Thayer, G. R. Faler, K. A. Zaklika, E. C. Blossey, and D. C. Neckers, Photooxygenations with Heterogeneous Sensitizers, *J. Photochem., 5,* 191 (1976).

13. A. P. Schaap, P. A. Burns, and K. Zaklika, Silica Gel-Catalyzed Rearrangement of an Endoperoxide to a 1,2-Dioxetane, *J. Am. Chem. Soc., 99,* 1270 (1977).

14. K. A. Zaklika, P. A. Burns, and A. P. Schaap, Enhanced Chemiluminescence from the Silica Gel-Catalyzed Decomposition of a 1,2-Dioxetane, *J. Am. Chem. Soc., 100,* 318 (1978).

15. K. A. Zaklika, A. L. Thayer, and A. P. Schaap, Substituent Effects on the Decomposition of 1,2-Dioxetanes, *J. Am. Chem. Soc., 100,* 4916 (1978).

16. A. P. Schaap and K. A. Zaklika, 1,2-Cycloaddition Reactions of Singlet Oxygen, In *Singlet Oxygen,* H. H. Wasserman and R. W. Murray (Eds), Academic Press, Inc., New York, P. 173 (1979).

17. A. P. Schaap, A. L. Thayer, K. A. Zaklika, and P. Valenti, Photooxygenations in Aqueous Solution with a Hydrophilic Polymer-Immobilized Photosensitizer, *J. Am. Chem. Soc., 101,* 4016 (1979).

18. K. A. Zaklika, T. Kissel, A. L. Thayer, A. P. Burns, and A. P. Schaap, Mechanisms of 1,2-Dioxetane Decomposition: The Role of Electron Transfer, *Photochem. Photobiol., 30,* 35 (1979).

19. K. A. Zaklika, B. Kaskar, and A. P. Schaap, Mechanisms of Photooxygenation.1. Substituent Effects on the 2 + 2 Cycloaddition of Singlet Oxygen to Vinyl Ethers, *J. Am. Chem. Soc., 102,* 386 (1980).

20. A. P. Schaap, K. A. Zaklika, B. Kaskar, and L. W. M. Fung, Mechanisms of Photooxygenation. 2. Formation of 1,2-Dioxetanes via 9,10-Dicyanoanthracene-Sensitized Electron-Transfer Processes, *J. Am. Chem. Soc., 102,* 389 (1980).

21. A. P. Schaap, S. Gagnon, and S. Anderson, Efficient Chemiluminescence from a Phenoxide-Substituted 1,2-Dioxetane, *J. Photochem., 17,* 104 (1981).

22. A. P. Schaap and S. Gagnon, Chemiluminescence from a Phenoxide-Substituted 1,2-Dioxetane: A Model for Firefly Bioluminescence, *J. Am. Chem. Soc., 104,* 3504 (1982).

23. A. P. Schaap, S. Gagnon, and K. A. Zaklika, Substituent Effects on the Decomposition of 1,2-Dioxetanes: A Hammett Correlation for Substituted 1,6-Diaryl-2,5,7,8-tetraoxabicyclo[4.2.0]octanes, *Tetrahedron Lett.,* 2943 (1982).

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LIST OF TABLES

LIST OF FIGURES

SUMMARY OF ARO-SUPPORTED RESEARCH (1972 - 1981)

Heterogeneous Photosensitizers for the Generation of Singlet Molecular Oxygen. Our ARO-supported research on photooxidation has resulted in the development of two polymer-immobilized photosensitizers for use in organic and aqueous solution[1]. These heterogeneous sensitizers have been particularly important to our work on the synthesis and isolation of the high-energy peroxides used for the chemiluminescence studies. Many other investigators have also reported these sensitizers to be synthetically useful in their work. Interest by other groups was sufficient to prompt the commercial preparation of these sensitizers by Hydron Laboratories, Inc., New Jersey.

The photosensitized formation of singlet oxygen ($^1O_2$) using various dyestuffs involves photochemical excitation of the sensitizer (dye) to its singlet excited state, intersystem crossing to the triplet state, energy transfer to ground state oxygen, and subsequent reaction of $^1O_2$ with an acceptor. However, there have been several limitations to the photooxidation method using sensitizers dissolved in the reaction solution: (1) the dye must be soluble in the reaction solvent, limiting the dye/solvent combinations which can be used; (2) the dye is often bleached over long reaction times; (3) the dye can interact with the substrates and/or the products, and (4) the separation of the dye from the products can be difficult.
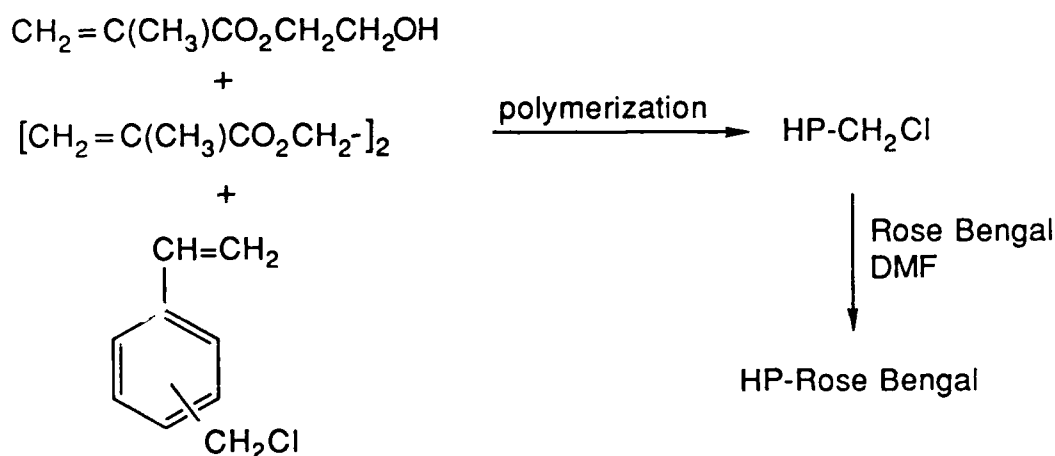
The heterogeneous sensitizers developed by our group in collaboration with D. C. Neckers (Bowling Green State University) obviate most of the difficulties of soluble sensitizers. The technique involves the chemical attachment of a sensitizer, Rose Bengal, to insoluble styrene-divinylbenzene copolymer beads. The polymer-immobilized photosensitizer (P-Rose Bengal or SENSITOX I) is prepared by refluxing a mixture of Rose Bengal and the beads in DMF. Quantum yield studies demonstrated that the efficiency for the formation of singlet oxygen by this sensitizer is 0.43.

chloromethylated styrene-divinylbenzene + Rose Bengal $\xrightarrow[\text{reflux}]{\text{DMF}}$ (P)-Rose Bengal copolymer beads

Rose Bengal
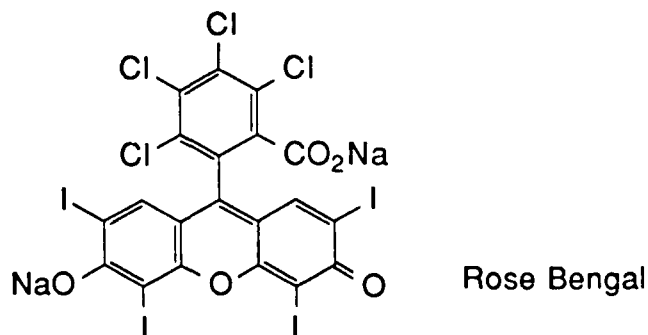
Heterogeneous sensitizers have a number of practical advantages over soluble sensitizers. These include (1) increased photostability of the dye on the polymer; (2) decreased secondary interaction of the photosensitizer with substrates or products; (3) utility in solvents in which the free dye is insoluble; (4) reuse of the sensitizer; (5) removal by simple filtration or sedimentation. The last feature implies, for example, that a continuous photooxidation process should be feasible, and that photooxygenations may be followed spectroscopically in regions normally masked by dye absorption.

Although the styrene-derived sensitizer was demonstrated to be useful in most organic solvents, we found it to be a poor photosensitizer in aqueous systems. The reason for this limited effectiveness is related to the observations that the hydrophobic polymer is not wetted by water, is difficult to suspend in aqueous media, and does not swell in water. A water-compatible heterogeneous sensitizer which extends advantages of the method to investigation of singlet oxygen reactions in aqueous systems was, therefore, developed in our laboratory.

A hydrophilic polymer was prepared by copolymerization of chloromethylstyrene, the mono-methacrylate ester of ethylene glycol, and the bis methacrylate ester of ethylene glycol as cross-linking agent. The resulting polymer was obtained in the form of beads (38-75 μ) and was heated with Rose Bengal in dry DMF at 60 $^{\circ}$C for 3 days to yield the hydrophilic sensitizer (HP-Rose Bengal or SENSITOX II) which is wetted by water and swells by 40% in this solvent.
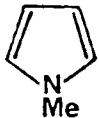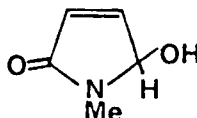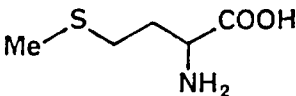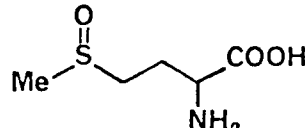
$$CH_2 = C(CH_3)CO_2CH_2CH_2OH$$
$$+$$
$$[CH_2 = C(CH_3)CO_2CH_2\text{-}]_2 \xrightarrow{\text{polymerization}} HP\text{-}CH_2Cl$$
$$+$$

$$\underset{CH_2Cl}{\overset{CH=CH_2}{\bigcirc}}$$

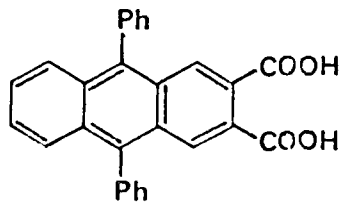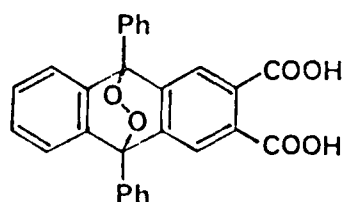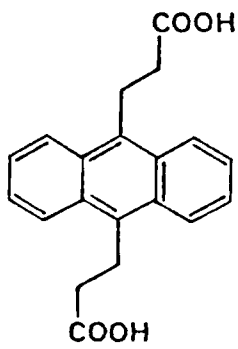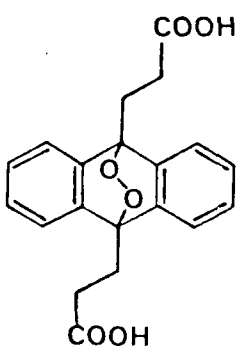HP-CH$_2$Cl $\xrightarrow[\text{DMF}]{\text{Rose Bengal}}$ HP-Rose Bengal

The effectiveness of HP-Rose Bengal in sensitizing singlet oxygen formation in water was illustrated by the successful photooxygenation of a number of substrates known to react with singlet oxygen in water. Typically, photooxidations were conducted with 0.01-0.1 M substrate in distilled water. Sodium hydroxide or hydrochloric acid was used to adjust pH where necessary. Suspensions of 5 to 20 mg/ml of HP-Rose Bengal were generally used, except in large-scale reactions where as little as 0.3 mg/ml was found adequate due to the longer light path in the apparatus. We have found that a 400-W sodium lamp (General Electric Lucalox) is particularly suited to this sensitizer. The bulk of the emission is at wavelengths absorbed by the dye and the absence of a significant UV component obviates the need for filter. Further, the lamp is energy efficient, has a long lifetime, and requires little cooling. The

results of the photooxidation experiments are presented in Table 1. The last entry indicates that the sensitizer is also synthetically useful in alcoholic solvents.

In view of the satisfactory behavior of HP-Rose Bengal in water, we were prompted to compare the effectiveness of this sensitizer with that of P-Rose Bengal in a variety of solvents. Photooxidation of tetramethylethylene using standard suspensions of the two sensitizers showed that both sensitizers behaved very similarly in polar solvents such as acetone, dichloromethane, or methanol. The quantum yield for singlet oxygen formation was determined for HP-Rose Bengal in methanol to be 0.48.

Table 1. Photooxygenations with HP-Rose Bengal

Substrate	Solvent	Product	Isolated Yield
	H_2O, pH 7		36%
	H_2O		85%
	H_2O, pH 9		89%
	H_2O, pH 8		95%
PhSMe	MeOH	PhSOMe	99%

1-Phospha-2,8,9-trioxaadamantane Ozonide: A Convenient Chemical Source of Singlet Molecular Oxygen. We have reported that addition of ozone to 1-phospha-2,8,9-trioxaadamantane (1) affords the relatively stable ozonide 2 which decomposes quantitatively to $^1O_2$ and phosphate[2]. Evolution of oxygen from this ozonide in the presence of a singlet oxygen substrate has been used to carry out oxygenation reactions. The rates of decomposition of the ozonide in CH_2Cl_2 were measured at a series of temperatures by following the oxygen evolution. These data were used to calculate the activation parameters for decompostion: E_a = 19.4 kcal/mol; log A = 11.5. The ozonide exhibits a half-life of 25 min at +11 $^\circ$C.
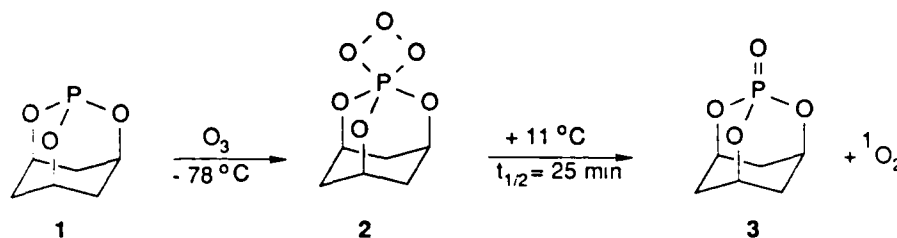
Silica Gel-Catalyzed Rearrangement of an Endoperoxide to a 1,2- Dioxetane. The spontaneous decomposition of suitably substituted 1,2-dioxetanes can result in the formation of electronically excited carbonyl products in high yield. However, most of these energy-rich compounds are not sufficiently stable at ambient temperature to have a reasonable shelf-life in a chemical light device. One solution to this problem has been found with our observation that a 1,2-dioxetane can be "stored" in the form of a crystalline, thermally-stable endoperoxide and the dioxetane produced in quantitative yield when needed[3]. The silica-catalyzed rearrangement is complete within a few seconds and subsequent decomposition of the dioxetane at ambient temperature provides intense chemiluminescence over a period of several hours.
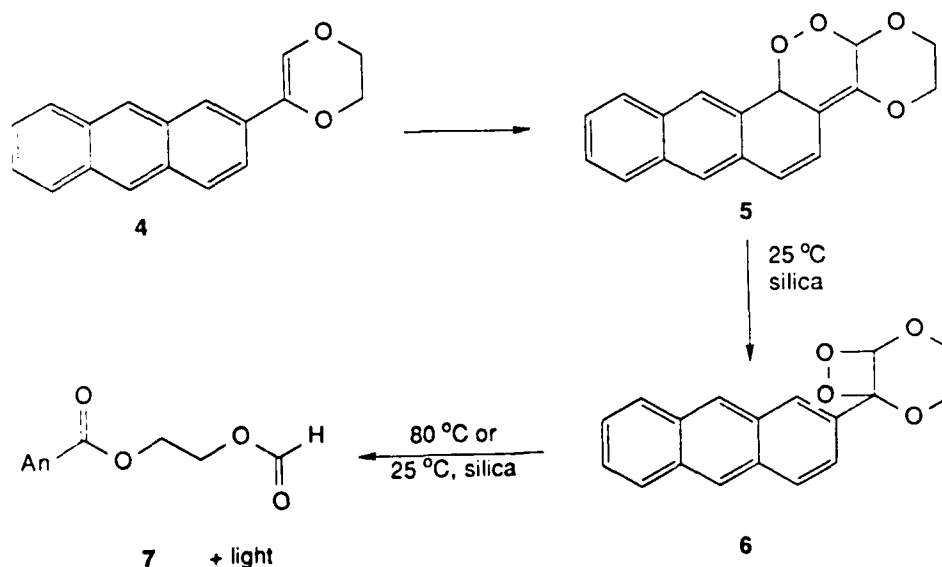
Photooxygenation of 2-(2-anthryl)-1,4-dioxene (4) in CH_2Cl_2 using polymer-immobilized Rose Bengal as sensitizer gave the endoperoxide **5**. However, unlike most dioxetanes which undergo spontaneous decomposition at ambient temperature, the endoperoxide is thermally stable exhibiting significant decomposition only upon heating for 12 h at 100 °C in o-xylene. However, upon treatment with silica gel at ambient temperature, a solution of the endoperoxide produces an intense bluish chemiluminescence with concomitant formation fo the cleavage product. We have shown that this luminescence is a result of two silica gel-dependent phenomena; the silica gel serves not only to effect a rearrangement of endoperoxide **5** to dioxetane **6**, but also to catalyze the decompostion of the dioxetane with higher chemiluminescence efficiency.

Enhanced Chemiluminescence from the Silica-Catalyzed Cleavage of Dioxetanes.

Our research on the silica-catalyzed rearrangement of **5** led to the first observation of enhanced chemiluminescence associated with a catalyzed decomposition of a stable dioxetane.[4] Thermolysis of the dianthryl-substituted 1,2-dioxetane **9** in o-xylene results in quantitative formation of the diester **10** and is accompanied by weak light emission. The rates of decompostion of **9**, the formation of **10**, and the emission of light are first-order and identical ($k = 6.44 \times 10^{-3}$ s^{-1} at 84.1 °C). The spectrum of the chemiluminescence was acquired by photon counting techniques, and is completely congruent with the fluorescence spectrum of **10** (Figure 1). The chemiluminescence efficiency for the formation of singlet excited **10** was found to be 0.7% at 84.1 °C.

However, addition of silica gel to an o-xylene solution of **9** leads to an immediate and spectacular increase in light intensity (as much as 10^4-fold at 25 °C with a 150-fold increase in rate. The kinetics of the catalyzed chemiluminescence were examined using stirred suspensions of silica gel in o-xylene. These results are presented in Figure 2. The integrated light emission per mole of 1,2-dioxetane **9** increases very rapidly with the weight of silica in suspension, unitl a plateau is reached corresponding to 136-fold enhancement of the observed chemiluminescence compared to the uncatalyzed decompositon of **9**. This result suggests that above 40 g/L of silica the chemiluminescence is derived entirely from the cleavage of **9** on the surface of the silica with increased efficiency for the formation of singlet excited **10**. While in o-xylene the singlet chemi-excitation efficiency is only 0.7%, in the presence of silica gel the efficiency for the production of singlet excited **10** by the catalytic reaction is 43%.
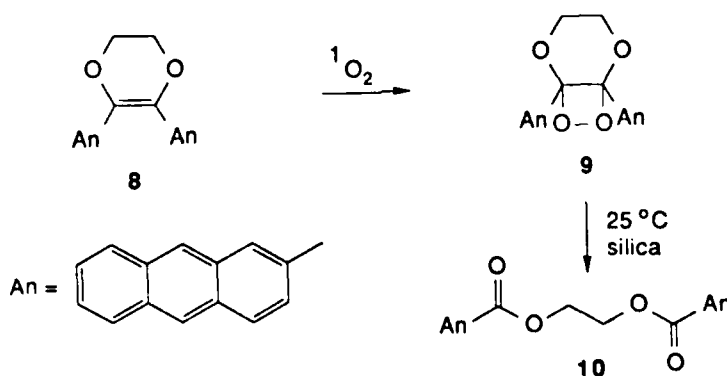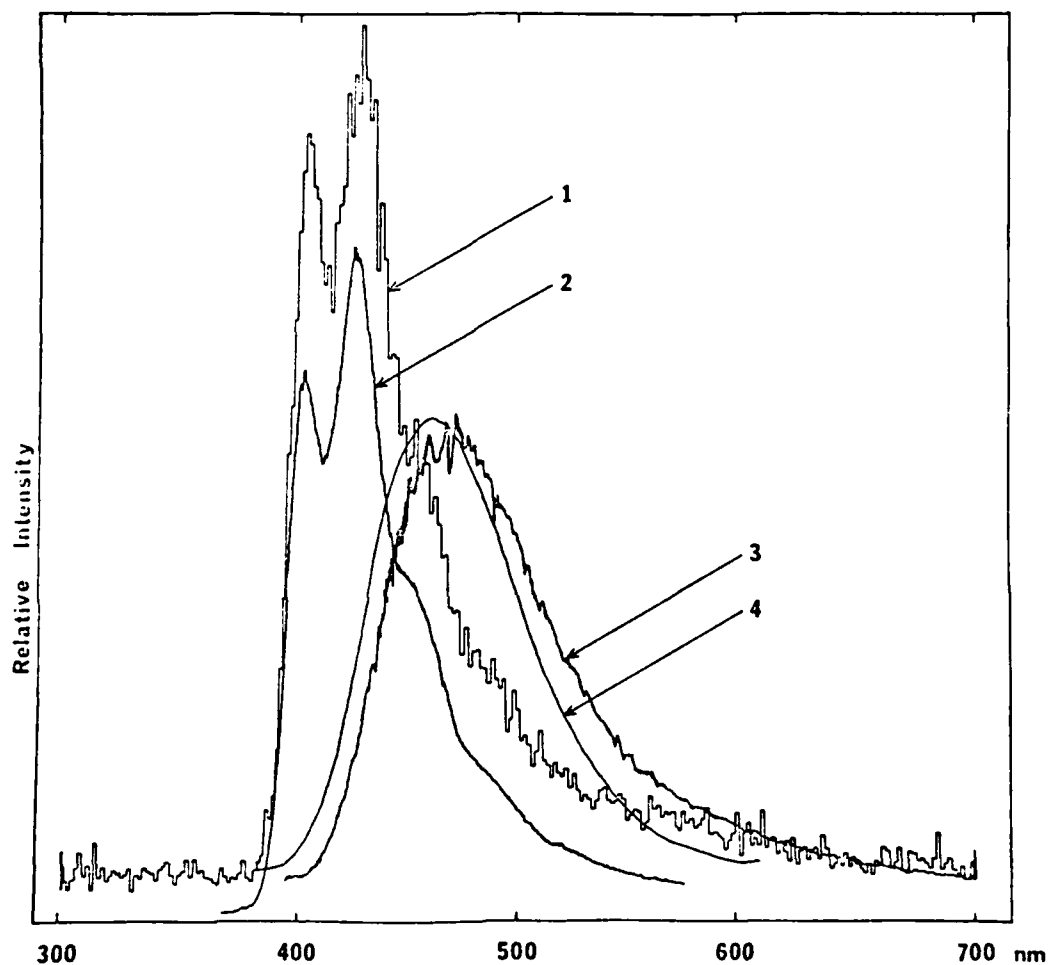
Figure 1. Emission spectra: (1) chemiluminescence from the thermolysis of dioxetane 9 in o-xylene at 55 °C; (2) fluorescence from 10 in o-xylene under the same conditions as curve 1; (3) chemiluminescence from the silica gel catalyzed decomposition of dioxetane 9 at 25 °C; (4) fluorescence of 10 adsorbed on silica gel in an o-xylene suspension.

Figure 2. Chemiluminescence from 1,2-dioxetane 9 in o-xylene with silica gel at 84 °C:
O, integrated light intensity per mole of 9 relative to that from the uncatalyzed reaction
(left-hand scale); ●, rate constant for the decay of chemiluminescence (right-hand
scale).

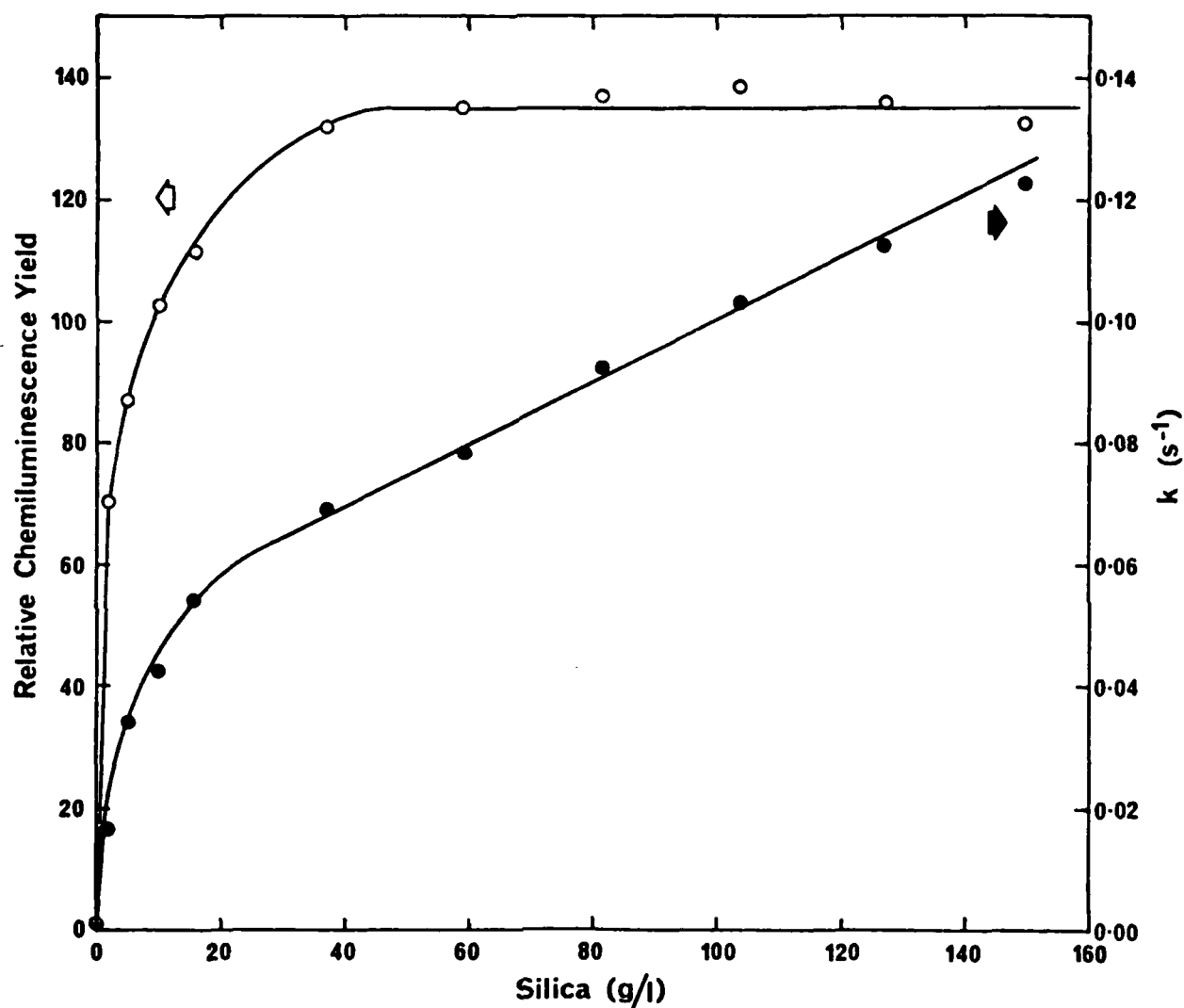Examination of chemiluminescence and fluorescence spectra has verified that the decomposition of dioxetane 9 and the light emission of singlet excited 10 occurs from the surface of the silica and not as a result of the action of some o-xylene-soluble catalytic entity present in the silica. Figure 1 shows that the fluorescence of 10 and the chemiluminescence from 9 are essentially superimposable with a significant red shift and loss of structure in the presence of silica. A comparison of this luminescence to the fluorescence of methyl 2-anthroate has shown that there is a significant component of eximer emission in the red-shifted chemiluminescence from 9 on silica.

An explanation for the silica-catalysis of the dioxetane luminescence involves an electron-transfer mechanism accelerated by the polar, protic surface of the silica gel. As a result of this hypothesis, we were led to investigate the effect of homogeneous catalysis by solvents[5]. The results of a study of the solvent effect on the chemiluminescent decomposition of 9 are shown in Table 2. In all cases, cleavage product 10 was the only detectable product. The first four entires show that the rate is insensitive to solvent polarity. The slight observed increases in the photon yields are probably due in most part to increases in the fluorescence efficiency of 10. These trends are negligible when compared with the last entires, which indicate a powerful catalytic effect of hydroxylic solvents with a calculated singlet chemiexcitation efficiency ($\emptyset_E$) for 9 in trifluoroethanol of 97%, compared to 0.7% in o-xylene. This is a 138-fold enhancement in efficiency in trifluoroethanol and more than twice the enhancement observed with silica.

We feel that the role of the various catalysts in promoting the enhanced chemiluminescence efficiency from anthryl-substituted dioxetanes 6 and 9 may be to activate an intramolecular electron-transfer process related to those observed for dioxetanes containing easily oxidized substituents. It is noteworthy that the chemiluminescence spectrum of 9 in trifluoroethanol closely resembles that of 9 on silica.

Table 2. Solvent Effects on the Decomposition of Dianthryl-Substituted 1,2-Dioxetane 9.

Solvent	k_{rel} (70°C)	rel photon yield[a]
methylcyclohexane	1.0	1.0
o-xylene	1.3	2.2 ($^1\emptyset_E$ = 0.7%)[b]
1,4-dioxane	1.1	3.4
acetonitrile	1.1	5.4
ethanol	3.8	66
2,2,2-trifluoroethanol	238	190 ($^1\emptyset_E$ = 97%)[b]

[a]Not corrected for change in fluorescence efficiency of 10. [b]Singlet chemiexcitation efficiency based on the Hastings[14]C light standard.

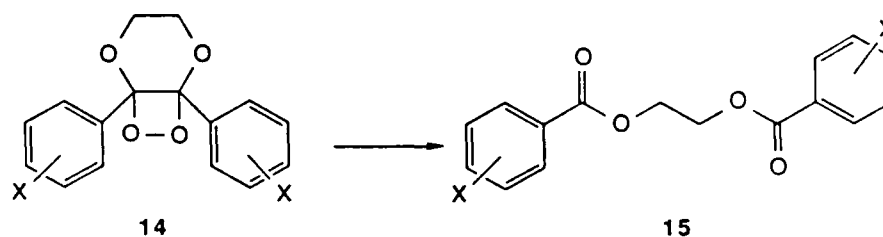<u>Substituent Effects on the Decomposition of 1,2-Dioxetanes. The Role of Electron-Transfer Processes in Efficient Chemiluminescence.</u> There is now unambiguous evidence from $^{18}O_2$ labeling experiments for the intermediacy of 1,2-dioxetanone 12a in firefly bioluminescence[6]. However, the properties of the biological system are in sharp contrast to those of simple, isolable 1,2-dioxetanes such as tetramethyl-1,2-dioxetane (TMD)[7]. While the bioluminescence is extremely efficient, producing essentially one singlet excited state molecule for every luciferin molecule that is oxidized, dioxetanes such as TMD yield only a fraction of a percent of the cleavage product in the $^1S^*$ state, although often giving significant yields of triplet excited carbonyl product. Further, the simple or "normal" dioxetanes are relatively stable with half-lives at ambient temperature of several days. The bioluminescent intermediate must, on the other hand, be quite unstable at ambient temperature as evidenced by the fact that the duration of the flash of the firefly luminescence can be as short as 40 ms. The contrast is one of high stability/low singlet chemiexcitation efficiency for simple dioxetanes and low stability/high singlet bio-excitation efficiency for the biological intermediates. Additional observations which provide some insight into the mechanism of the excitation process in the firefly luminescence are those of White and coworkers[9] on the <u>in vitro</u> bioluminescence produced by various substituted luciferins 11. It was found that although all of the substrates react with oxygen in the presence of the luciferase to give the putative dioxetanones, only in the systems involving dioxetanones 12a and 12c is luminescence observed. Our study of analogously substituted dioxetanes described below now provides a basis for understanding these early oberservations of White with the key factor for luminescence being the oxidation potential of the moiety attached t the dioxetanone or dioxetane ring. An electron-transfer mechanism has been proposed for the efficient generation os singlet excited states from 1,2-dioxetanes[5,10]. The results are in accord with those of White and suggest a similar mechanism for the firefly bioluminescence.

MECHANISM OF FIREFLY BIOLUMINESCENCE

luciferin 11

a) X = O⁻ (bioluminescent)
b) X = OCH₃ (dark)
c) X = NH₂ (bioluminescent)

12
dioxetane intermediate

13

+ CO_2 + light
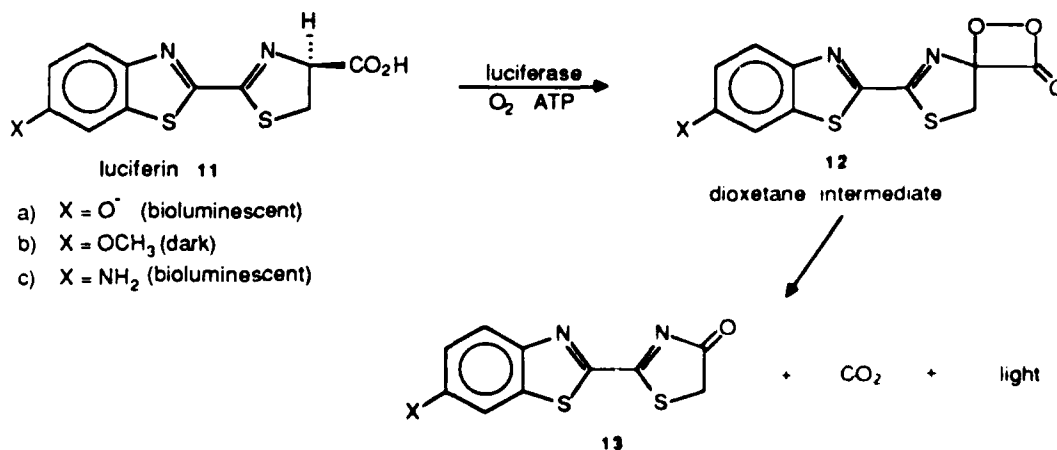
Although a large number of dioxetanes have been synthesized in the last 10 years, the wide variation in structural type has made comparisons difficult, and hampered the detection of mechanistic trends. To circumvent this problem, we carried out a study based on systematic variation of the substitutents in a series of 1,6-diaryl-2,5,7,8-tetraoxabicyclo[4.2.0]octanes[10]. In addition to an examination of substitutent effects, our work has extended to a study of solvent effects, and to the application of energetic criteria. These studies have provided evidence for two types of mechanisms operative in dioxetane decomposition: (1) a process which is relatively insensitive to both aryl-substitutents and solvent polarity and which results in low singlet chemiexcitation efficiencies and (2) a solvent-sensitive mechanism which is strongly dependent on the oxidation potential of the substitute and results in efficient chemiluminescence.

Dioxetanes **14** were prepared by low temperature photooxygenation of the corresponding olefins using polymer-immobilized Rose Bengal. These dioxetanes were fully characterized by ^{1}H and ^{13}C NMR spectroscopy, and thermolyzed to the expected diesters **15**. The thermolyses were cleanly first order with no observed dark catalysis by solvent impurities. Excluding **14a**, dioxetanes **14b-m** are all stable, isolable compounds that exhibit relatively constant Arrhenius activation energies for decomposition to (23.6-25.1 kcal/mol). Esters **15b-m** are not detectably fluorescent so that singlet and triplet yields were determined from Stern-Volmer plots using 9,10-diphenylanthracene and 9,10-dibromoanthracene as energy acceptors. Our results have shown that "normal" dioxetanes produce low yields (<0.1%) of singlet excited with modest yields of triplets (see Table 3). Dioxetanes were found to obey a Hammett relationship for the rates of decomposition against σ+ values with ρ = -0.2 and r = 0.91 (Figure 3) suggesting a common mechanism of decompostion. We interpret the small ρ value associated with these dioxetanes as support for the biradical mechanism rather than a concerted mechanism in which the developing polarized carbonyl group would be expected to have more sensitivity to substituents.

14

15

a, X = p-NMe₂
b, X = p-OMe
c, X = H
d, X = p-OiPr
e, X = p-OH
f, X = p-NHAc
g, X = p-OPh
h, X = p-Me
i, X = m-OMe
j, X = p-Ph
k, X = p-Cl
l, X = m-Cl
m, X = m-CF₃

Dioxetane **14a** (X = NMe$_2$) clearly does not follow the Hammett substituent relationship (Figure 3) and decomposes by an alternate mechanism. In additon to being relatively unstable exhibiting a brilliant bluish luminescence even at -20 $^{\circ}$C, **14a** shows a 1000-fold increase in the singlet chemiexcitation efficiency (Table 3). Ester **15a** is fluorescent and the chemiluminescence of **14a** mathches this fluorescence allowing direct evaluation of the singlet efficiency in this case. Further, dioxetane **14a** exhibits a marked solvent effect (Table 4) that is not observed for normal dioxetanes suchs as **14c**.

Table 3. Activation Parameters, Rates of Decompostion, and Chemiluminescence Efficiencies for 1,2-Dioxetanes **14** in o-Xylene or Toluene.

Dioxetane (X)	E$_a$ (kcal/mol)	Log A	k$_{rel}$ (25°C)	$^1\varnothing_E$[b]	$^3\varnothing_E$[c]
14a (p-NMe$_2$)	19.3	10.99	382	22%[d]	------
14b (p-OMe)	24.0	12.38	3.51	0.024%[e]	5.0%
14c (H)	24.8	12.39	1.00[a]	0.019%[e]	6.8%

[a]Corresponding to a rate constant of 1.62 x 10^{-6} s^{-1} at 25°C. [b]Chemiexcitation efficiency for the formation of singlet excited **15**. [c]Chemiexcitation efficiency for the formation of triplet excited **15**. [d]At 25 $^{\circ}$C in toluene. [e]At 95 $^{\circ}$C in o-xylene.

Table 4. Solvent Effects on the Decomposition of Dimethylamino-Substituted Dioxetane **14a** at 25°C.

Solvent	k$_{rel}$	E$_a$ (kcal/mol)	E$_T$(kcal/mol)
toluene	1.0[a]	19.3	33.9
1,2-dimethoxyethane	2.5	18.2	38.6
acetophenone	25	16.0	41.3
1,2-dicloroethane	27	-----	41.9
N, N-dimethylformamide	57	16.6	43.8
acetonitrile	81	16.0	46.0
propylene carbonate	188	14.8	46.6

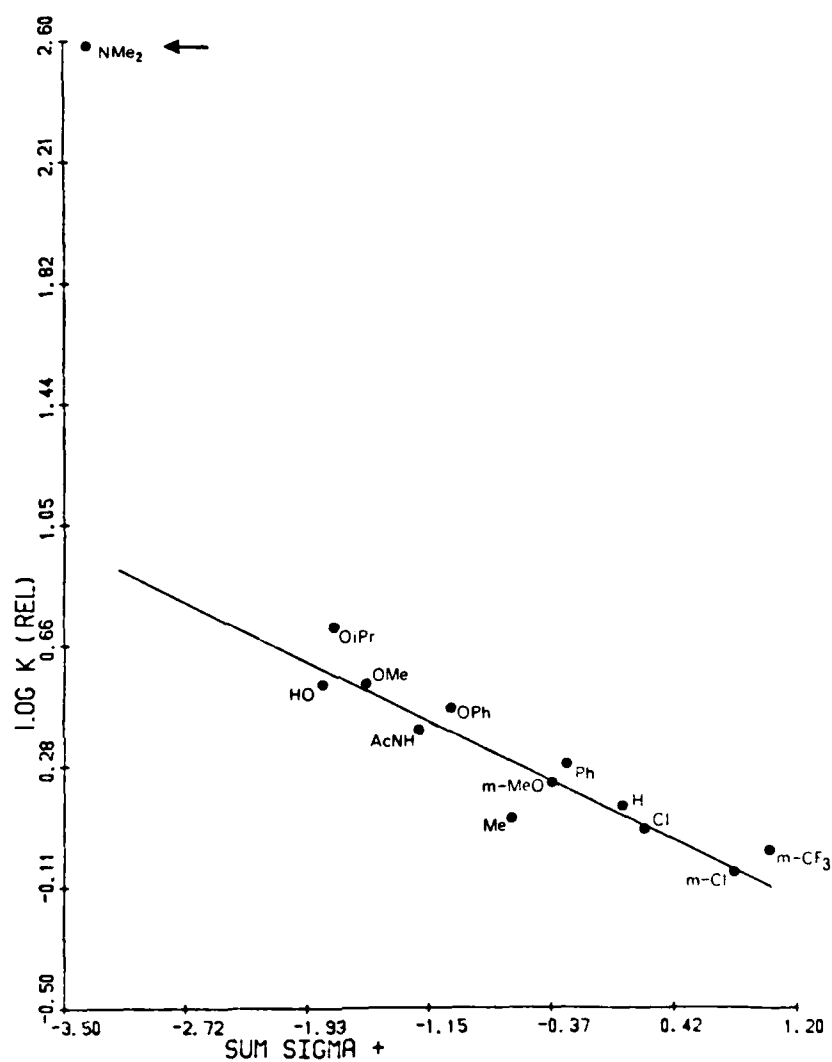[a]Corresponding to a rate constant of 6.15 x 10^{-4} s^{-1} at 25°C.

Figure 3. Hammett plot for the logarithm of the rate of decomposition of dioxetanes 14 against the sum of the substituent constants σ^+: all substituents are para unless otherwise indicated.

Chemical Triggering of a Chemiluminescent 1,2-Dioxetane. As part of our study of substitutent effects on the stability and chemiluminescence efficiency of dioxetanes, we discovered that deprotonation of a phenolic substitutent converts a stable and inefficiently luminescent dioxetane into one which exhibits properties characteristic of bioluminescent intermediates[11]. Our approach to this problem was based on the proposed electron-tranfer mechanism involving oxidation of the substituent and the fact that the phenoxide ion is more easily oxidized than phenol. Additionally, the observations of White on the in vitro bioluminescence from substituted luciferins suggested that a phenoxide-substituted dioxetane might show interesting properties.

Dioxetane **17a** derived from the addition of singlet oxygen to 2-(4-hydroxy-phenyl)-3-phenyl-1,4-dioxene **16a** is quite stable and decomposes with a low singlet chemiexcitation efficiency that is typical of many simple 1,2-dioxetanes (Table 5). However, treatment of **17a** in toluene at -30°C with the hindered non-nucleophilic base, $(Me_3Si)_2MeCOLi$, produces dioxetane **17b** which decomposes with a flash of brilliant bluish luminescence. It is apparent that the easily oxidized-phenoxide subsituent of **17b** dramatically alters the properties of this dioxetane. The activation energy for cleavage of **17b** is 13.4 kcal/mol with a calculated half-life at 25 °C of only 46 ms. The relative rate of decompositon of **17b** vs. **17a** (k_{O^-}/k_{OH}) at 25 °C is 4.5 x 10^6. There is also a corresponding increase in the chemiexcitation efficiency by a factor of approximately 3 orders of magnitude. The properties of **17b** closely parallels those exhibited by the firefly intermediate with high efficiency and extremely low stability. A comparison of the efficienceies and stabilities of dioxetanes **17a** and **17b** with those of the biological intermediates **12b** and **12a**, respectively, suggests a possible control mechanism for the rapid flashing of the firefly bioluminescence. These results also provided further evidence for an intramolecular electron-transfer mechanism in efficient chemiexcitation from suitably substituted 1,2-dioxetanes.
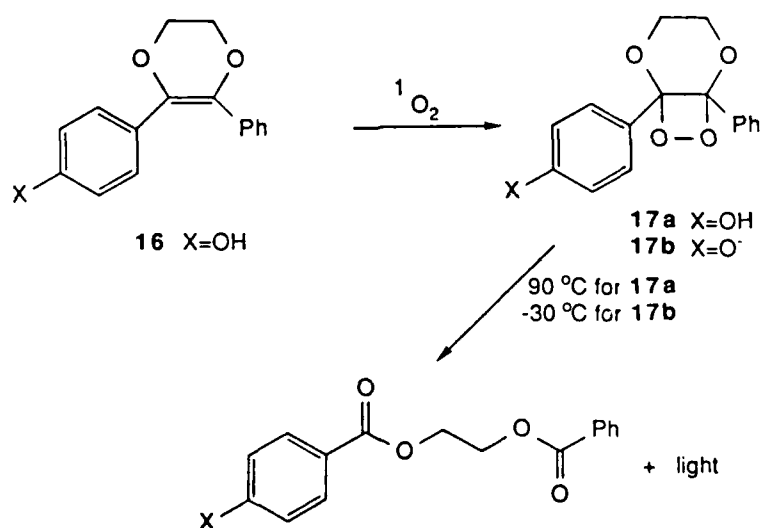
Table 5. Activation Parameters, Rates of Decomposition and Chemiluminescence Efficiencies for 1,2-Dioxetanes 17.

Dioxetane (X)	E_a (kcal/mol)	Log A	rel rate at $25^{\circ}C$	half-life at $25^{\circ}C$	$^1\emptyset_E$	$^3\emptyset_E$
14c (H)	24.8	12.39	0.48	129 h	0.02%	6.8%
17a (OH)	24.4	12.38	1.00[a]	57 h	0.01%[b]	1.5%[c]
17b (O⁻)	13.4	11.0	4.5×10^6	46 ms	>20%[b,d]	-------

[a]Corresponding to a rate constant in o-xylene at $25^{\circ}C$ of $3.40 \times 10^{-6}s^{-1}$. [b]Chemiluminescence efficiency for the formation of singlet excited $. [c]Chemiluminescence efficiency for the formationof triplet excited $. Efficiencies are based on a calibraiton with the Hastings ^{14}C radioactive light standard. [d]At $-30^{\circ}C$ in toluene.

LITERATURE REFERENCES

1. (a) E. C. Blossey, D. C. Neckers, A. L. Thayer, and A. P. Schaap,*J. Am. Chem. Soc.*, 95, 5820 (1974). (b) A. P. Schaap, A. L. Thayer, E. C. Blossey,and D. C. Neckers, *J. Am. Chem. Soc.*, 97, 7110 (1975). (c) A. P. Schaap, A. L. Thayer, G. R. Faler, K. A. Zaklika, E. C. Blossey, and D. C. Neckers, *J. Photochem.*, 5, 191 (1976).

2. A. P. Schaap, K. Kees, and A. L. Thayer, *J. Org. Chem.*, 40, 1185 (1975).

3. A. P. Schaap, P. A. Burns, and K. Zaklika, *J. Am. Chem. Soc.*, 99, 1270 (1977).

4. K. A. Zaklika, P. A. Burns, and A. P. Schaap, *J. Am. Chem. Soc.*, 100, 318 (1978).

5. K. A. Zaklika, T. Kissel, A. L. Thayer, A. P. Burns, and A. P . Schaap, *Photochem. Photobiol.*, 30, 35 (1979).

6. O. Shimamura and F. H. Johnson, *Photochem. Photobiol.*, 30, 89 (1979).

7. (a) P. D. Bartlett and M. Landis in "Singlet Oxygen," H. H. Wasserman and R. W. Murray, Eds., Academic Press: New York, 1979, Chapter7. (b) T. Wilson, MTP Int. *Rev Sci., Phys, Chem., Ser 2*, 9, 265 (1976).

8. J. E. Lloyd in "Bioluminescence in Action," P. J. Herring, Ed., Academic Press: New York, 1978, p.264.

9. E. H. White, E. Rapaport, T. A. Hopkins, and H. H. Seliger, *ibid.*, 91, 2178 (1969).

10. (a) K. A. Zaklika, A. L. Thayer, and A. P. Schaap, *J. Am. Chem. Soc.*, 100, 4916 (1978). (b) A. P. Schaap, S. Gagnon, and K. A. Zaklika,*Tetrahedron Lett.*, 2943 (1982).

11. (a) A. P. Schaap, S. Gagnon, and S. Anderson, *J. Photochem.*, 17, 104 (1981). (b) A. P. Schaap, S. Gagnon, *J. Am.Chem. Soc.*, 104, 3504 (1982).

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